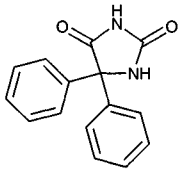
ronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Phenytoin



Molecular formula: $C_{15}H_{12}N_2O_2$
Molecular weight: 252.27
CAS Registry No.: 57-41-0, 630-93-3 (sodium salt)
Merck Index: 7475
Lednicer No.: 1 246

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 100 μL 30 mg/L IS in water + 200 μL 25% saturated ammonium acetate, mix. Add the sample to the reservoir of a primed 4 mm/1 mL Empore C8 SPE disk cartridge suspended in a test tube (16 × 100 mm). Force the liquid then 500 μL water through the disk by centrifuging at 100-120 g for 5 min. Suspend disk cartridge in a tube, elute the drug with 100 μL MeCN and 300 μL water. Combine the eluates, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2 30 μm Permaphase ETH (DuPont)
Column: 250 × 4.6 Zorbax Stable-Bond CN
Mobile phase: MeCN:MeOH:acetic acid:triethylamine: water 15:12.5:0.1:0.06:72.5 (Connect a 250 × 4.6 column dry packed with 37-53 μm silica gel (Whatman) as a mobile-phase saturating column between the pump and the injector .)
Column temperature: 50
Flow rate: 1.2
Injection volume: 50
Detector: UV 214

CHROMATOGRAM
Retention time: 10.5
Internal standard: cyheptamide (14)
Limit of detection: 20-35 ng/mL

OTHER SUBSTANCES
Extracted: carbamazepine, carbamazepine diol, carbamazepine epoxide, lamotrigine, 5-(p-hydroxyphenyl)-5-phenylhydantoin

Simultaneous: acetaminophen, N-acetylprocainamide, amikacin, caffeine, chlordiazepoxide, clonazepam, desmethylchlordiazepoxide, desmethyldiazepam, diazepam, digoxin, disopyramide, erythromycin, ethosuximide, felbamate, flurazepam, gabapentin, gentamicin, lidocaine, methotrexate, nitrazepam, oxazepam, phenylethylmalonamide, phenobarbital, primidone, quinidine, salicylate, temazepam, theophylline, tobramycin, valproic acid, vancomycin

KEY WORDS
serum; SPE

REFERENCE
Lensmeyer,G.L.; Gidal,B.E.; Wiebe,D.A. Optimized high-performance liquid chromatographic method for determination of lamotrigine in serum with concomitant determination of phenytoin, carbamazepine, and carbamazepine epoxide, *Ther.Drug Monit.*, **1997**, *19*, 292–300.

SAMPLE
Matrix: blood
Sample preparation: Mix 500 μL plasma with 500 μL MeCN and 2 μg IS for 30 s, centrifuge at 2700 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeCN:MeOH:10 mM pH 7.4 phosphate buffer 15:35:50
Column temperature: 25
Flow rate: 1
Detector: UV 219

CHROMATOGRAM
Internal standard: 2-hydroxy-2-ethyl-2-phenylacetamide
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: carbamazepine, clonazepam, ethosuximide, D,L-2-hydroxy-2-ethyl-2-phenylpropionamide (HEPP), phenobarbital, primidone

KEY WORDS
rat; plasma

REFERENCE
Martínez de Muñoz,D.; Arenas,R.; Chávez González,O. Liquid chromatographic assay in plasma of one of the members of a new series of anticonvulsants: D,L-3-hydroxy-3-ethyl-3-phenylpropionamide, *J.Chromatogr.B*, **1996**, *678*, 377–383.

SAMPLE
Matrix: blood
Sample preparation: Add 200 μL 2 μg/mL thymol in MeCN to 200 μL serum, vortex for 10 s, centrifuge at 7000 g for 5 min, inject 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Resolve C18-5 (Waters)
Mobile phase: MeCN:isopropanol:50 mM pH 3.0 phosphate buffer 25:15:60
Column temperature: 30
Flow rate: 0.7
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 7.5
Internal standard: thymol (18.5)

OTHER SUBSTANCES
Extracted: ethosuximide, primidone, phenobarbital, carbamazepine, valproic acid

KEY WORDS
human; plasma

REFERENCE
Kondo,K.; Nakamura,M.; Nishioka,R.; Kawai,S. Direct method of determination of valproic acid in serum by high performance liquid chromatography, *Anal.Sci.*, **1985**, *1*, 385–387.

SAMPLE
Matrix: blood
Sample preparation: Dilute 20 μL serum with 100 μL pH 3.7 phosphate buffer, shake vigorously for 10 s, add to a 45 μL PTFE column packed with 50 μm ODS-silica (Asahi Chemicals, Tokyo) (Extrashot-ODS device), wash with 100 μL water, elute with 130 μL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 7 μm Hibar LiChrosorb RP-18
Mobile phase: MeCN:MeOH:pH 4.4 potassium phosphate buffer 14:21:65
Flow rate: 1
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 15.9

OTHER SUBSTANCES
Extracted: carbamazepine, phenobarbital

KEY WORDS
SPE

REFERENCE
Kouno,Y.; Ishikura,C.; Homma,M.; Oka,K. Extrashot-ODS, a syringe-type minicolumn sample injector for a reversed-phase high-performance liquid chromatographic column. Application to antiepileptics in human sera, *J.Chromatogr.B*, **1997**, *695*, 349–353.

SAMPLE
Matrix: blood, milk
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL water and MeOH: water 20:80. Add 80 μg/mL mephenytoin solution to 1 mL human breast milk or plasma, add 5 mL 0.5% pH 6.0 KH_2PO_4, mix briefly, add the sample to the SPE cartridge, elute with 5 mL MeOH, evaporate the eluate to dryness, dissolve the residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Develosil C8-5 (Nomura Chemicals)
Mobile phase: MeCN:0.5% KH_2PO_4 buffer 30:70 (The pH of mobile phase was adjusted to 4.5 with 50% H_3PO_4.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 9
Internal standard: mephenytoin (13)

KEY WORDS
cord blood plasma; human breast milk; maternal plasma; plasma; human; SPE

REFERENCE
Shimoyama,R.; Ohkubo,T.; Sugawara,K.; Ogasawara,T.; Ozaki,T.; Kagiya,A.; Saito,Y. Monitoring of phenytoin in human breast milk, maternal plasma and cord blood plasma by solid-phase extraction and liquid chromatography, *J.Pharm.Biomed.Anal.*, **1998**, *17*, 863–869.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. Inject a 5-20 μL aliquot onto the column with mobile phase A or B. Urine. Inject a 5 μL aliquot onto the column with mobile phase C.

HPLC VARIABLES
Column: 100 × 4.6 5-10 μm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeCN:20 mM pH 6.9 phosphate buffer 19:81 (A) or Gradient. MeCN:20 mM pH 6.9 phosphate buffer from 5:95 to 20:80 over 2 min, to 25:75 over 2 min, to 30:70 over 4 min, to 50:50 over 2 min, maintain at 50:50 for 10 min (B) or Gradient. MeCN:20 mM pH 6.9 phosphate buffer 14:76 for 5 min, to 25:75 over 1 min, to 30:70 over 2 min, to 50:50 over 3 min, maintain at 50:50 for 6 min (C)
Flow rate: 1
Injection volume: 5 (A, C), 20 (B)
Detector: UV 254 (serum); UV 230 (urine)

CHROMATOGRAM
Retention time: 2.88 (serum, A), 11.5 (serum, B), 13.5 (urine, C)
Limit of detection: 2 ng (urine)

OTHER SUBSTANCES
Simultaneous: acetaminophen (B), barbital (B), carbamazepine (B,C), phenobarbital (B,C), phenytoin (C), primidone (B), sulfapyridine (B)
Also analyzed: metabolites

KEY WORDS
serum

REFERENCE
Ambrose,D.L.; Fritz,J.S. High-performance liquid chromatographic determination of drugs and metabolites in human serum and urine using direct injection and a unique molecular sieve, *J.Chromatogr.B*, **1998**, *709*, 89–96.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 16.288

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4 5 μm Phenyl Hypersil
Mobile phase: MeOH:buffer 35:65 (Buffer was 25 mM aqueous potassium phosphate monobasic solution adjusted to pH 3.8 with phosphoric acid.)
Column temperature: 50
Flow rate: 1.1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: fosphenytoin

REFERENCE
Narisawa,S.; Stella,V.J. Increased shelf-life of fosphenytoin: solubilization of a degradant, phanytoin, through complexation with $(SBE)_{7m}$-β-CD, *J.Pharm.Sci.*, **1998**, *87*, 926–930.

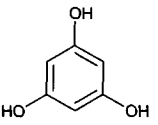
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Spherisorb ODS-2
Mobile phase: MeCN:water 40:60
Flow rate: 0.8
Detector: UV 228

CHROMATOGRAM
Retention time: 8

REFERENCE
Mithani,S.D.; Bakatselou,V.; TenHoor,C.N.; Dressman,J.B. Estimation of the increase in solubility of drugs as a function of bile salt concentration, *Pharm.Res.*, **1996**, *13*, 163–167.

Phloroglucinol



Molecular formula: $C_6H_6O_3$
Molecular weight: 126.11
CAS Registry No.: 108-73-6
Merck Index: 7482

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject

a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

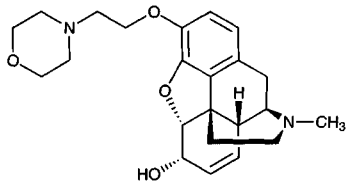
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85: 15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 202.8

CHROMATOGRAM
Retention time: 4.172

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Pholcodine



Molecular formula: $C_{23}H_{30}N_2O_4$
Molecular weight: 398.50
CAS Registry No.: 509-67-1
Merck Index: 7484
Lednicer No.: 1 287

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

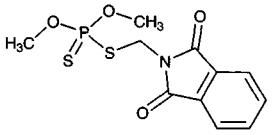
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85: 15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 211.1

CHROMATOGRAM
Retention time: 2.687

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Phosmet



Molecular formula: $C_{11}H_{12}NO_4PS_2$
Molecular weight: 317.33
CAS Registry No.: 732-11-6
Merck Index: 7492

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 150 mg phosmet in 5 mL THF, add 10 mL 1.3 mg/mL diphenylamine in THF, make up to 50 mL with n-hexane, add 1 g anhydrous sodium sulfate, mix well, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 2 MicroPak CN-10
Mobile phase: n-Hexane:THF 8:92
Flow rate: 0.83
Injection volume: 5
Detector: UV 280

CHROMATOGRAM
Retention time: 6
Internal standard: diphenylamine (2)

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Dulak,K.; Jonas,F. Determination of phosmet by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *396*, 433–436.

SAMPLE
Matrix: formulations
Sample preparation: Dilute formulation 100-fold with MeOH, centrifuge at 1250 g for 10 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 30 × 4.6 3 μm P-E 3 × 3 C18 (Perkin-Elmer)
Mobile phase: MeCN:water 85:15
Flow rate: 2
Injection volume: 10
Detector: UV 229

CHROMATOGRAM
Retention time: 0.31

Limit of detection: 80 pg

OTHER SUBSTANCES
Also analyzed: amitraz (UV 313), chlorpyrifos (UV 313), coumaphos (UV 313), crotoxyphos (UV 229), permethrin (UV 229)

REFERENCE
Rice,L.G. Rapid separation of pesticides by high-performance liquid chromatography with 3-μm columns, *J.Chromatogr.*, **1984**, *317*, 523–526.

SAMPLE
Matrix: solutions
Sample preparation: Equilibrate column A with 10 mL MeCN and 10 mL water (pH 7). Pump 200 mL drinking water through column A at 3 mL/min, back flush contents of column A onto column B with the mobile phase and start the gradient.

HPLC VARIABLES
Column: A 10 × 2.1 5 μm RP-18 octadecylsilica (E. Merck); B 150 × 4.6 5 μm Nucleosil C18
Mobile phase: Gradient. MeCN:water from 40:60 to 60:40 over 15 min
Injection volume: 200000
Detector: UV 254

CHROMATOGRAM
Retention time: 13.9
Limit of detection: 0.2 ng/mL

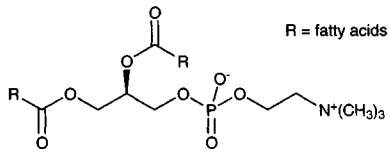
OTHER SUBSTANCES
Extracted: azinphos-methyl, carbaryl, parathion-methyl, azinphos-ethyl, fenitrothion, parathion, diazinon

KEY WORDS
drinking water; column-switching

REFERENCE
Driss,M.R.; Hennion,M.-C.; Bouguerra,M.L. Determination of carbaryl and some organophosphorus pesticides in drinking water using on-line liquid chromatographic preconcentration techniques, *J.Chromatogr.*, **1993**, *639*, 352–358.

Phosphatidylcholine

Merck Index: 5452



SAMPLE
Matrix: amniotic fluid
Sample preparation: Precipitate phospholipids from 5 mL amniotic fluid with cold acetone. Dissolve the precipitate in 30 μL chloroform, remove a 20 μL aliquot and add it to 40 μL chloroform:MeOH 2:1, inject a 10-30 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 10 μm SI 60 silica (Merck)
Column: 250 × 4.6 10 μm LiChrosorb DIOL
Mobile phase: Gradient. A was MeCN:water 80:20. B was MeCN. A:B 12.5:87.5 for 4.5 min, to 75:25 over 6.5 min.
Column temperature: 35
Flow rate: 2

Injection volume: 10-30
Detector: UV 203

CHROMATOGRAM
Retention time: 13

OTHER SUBSTANCES
Extracted: phosphatidyl glycerol, phosphatidyl inositol, phosphatidyl serine, phosphatidyl ethanolamine, sphingomyelin, lysolecithin

KEY WORDS
mobile phase temperature 40°

REFERENCE
Briand,R.L.; Harold,S.; Blass,K.G. High-performance liquid chromatographic determination of the lecithin/sphingomyelin ratio in amniotic fluid, *J.Chromatogr.*, **1981**, *223*, 277–284.

SAMPLE
Matrix: amniotic fluid
Sample preparation: Centrifuge amniotic fluid, remove a 5-10 mL aliquot and add it to an equal volume of MeOH, shake for 30 s, add twice the volume of chloroform, shake for 30 s, centrifuge at 1100 g for 5 min. Remove the lower chloroform layer and filter (Whatman GFF glass fiber), evaporate to dryness under a stream of nitrogen at 45°, add ice-cold acetone. Dry the precipitate thoroughly, take it up in 35 μL chloroform:MeOH 95:5, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: Corasil
Column: 300 × 4 10 μm μPorasil
Mobile phase: Chloroform:MeOH:water 178.5:64:5
Flow rate: 2
Injection volume: 25
Detector: RI

CHROMATOGRAM
Retention time: 20
Limit of detection: 3.7 μM

OTHER SUBSTANCES
Extracted: phosphatidyl glycerol, phosphatidyl inositol, phosphatidyl serine, phosphatidyl ethanolamine, sphingomyelin

KEY WORDS
normal phase

REFERENCE
Paton,R.D.; McGillivray,A.I.ir,T.F.; Whittle,M.J.; Whitfield,C.R.; Logan,R.W. HPLC of phospholipids in biological fluids --application to amniotic fluid for the prediction of fetal lung maturity, *Clin.Chim.Acta*, **1983**, *133*, 97–110.

SAMPLE
Matrix: amniotic fluid
Sample preparation: Centrifuge amniotic fluid, remove a 1.5 mL aliquot and add it to 1.5 mL MeOH, vortex for 30 s, add 6 mL chloroform, vortex for 30 s, centrifuge at 1500 g for 10 min. Remove the lower chloroform layer and evaporate it to dryness under a stream of nitrogen at 50°, chill the residue in a deep freeze for 10 min, add ice-cold acetone. Dry the precipitate thoroughly, take it up in 20 μL 22 μM gamma-capryloyl lysolecithin in chloroform:MeOH 2:1, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm SI 60 silica (Merck)

Column: 125 × 4.6 5 μm DIOL (Merck)
Mobile phase: Gradient. A was MeCN. B was MeCN:water 3.5:1. A:B 88:12 for 4.2 min, to 25: 75 over 8 min.
Column temperature: 38
Flow rate: 2
Injection volume: 10
Detector: UV 203

CHROMATOGRAM
Retention time: 13
Internal standard: gamma-capryloyl lysolecithin (16)
Limit of detection: 500 nM

OTHER SUBSTANCES
Extracted: phosphatidyl glycerol, phosphatidyl inositol, phosphatidyl serine, phosphatidyl ethanolamine, sphingomyelin

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KEY WORDS
mobile phase temperature 40°

REFERENCE
Andrews,A.G. Estimation of amniotic fluid phospholipids by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *336*, 139–150.

SAMPLE
Matrix: bile
Sample preparation: Shake 2 mL bile and 8 mL isopropanol, centrifuge. Purify 100 μL on a 10 × 10 cm Kieselgel 60 F254 TLC plate (Merck) by eluting with chloroform:MeOH:benzene: ammonia 65:30:10:6 (Caution! Benzene is a carcinogen!), visualize using iodine. Scrape off the band, elute with 5 mL chloroform:MeOH 4:3. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm mod HS-3 C18 (Perkin-Elmer)
Mobile phase: MeCN:MeOH:water 3:90:8 containing 20 mM choline chloride
Column temperature: 50
Flow rate: 1 for 2 min, increase to 2 over 20 min, maintain at 2 for 10 min
Injection volume: 10
Detector: UV 205

CHROMATOGRAM
Retention time: 12-25

REFERENCE
Cantafora,A.; Di Biase,A.; Alvaro,D.; Angelico,M.; Marin,M.; Attili,A.F. High performance liquid chromatographic analysis of molecular species of phosphatidylcholine--development of quantitative assay and its application to human bile, *Clin.Chim.Acta*, **1983**, *134*, 281–295.

SAMPLE
Matrix: bulk
Sample preparation: Emulsify 150 μg phosphatidylcholine in 8 mL 100 mM pH 5.5 acetate buffer and 1.5 mL 1 M calcium chloride, add 100 μg cabbage phospholipase D, add 4 mL diethyl ether, stir at rom temperature for 20 h, add 3 mL 500 mM EDTA, extract with chloroform/ MeOH. Dissolve the extracted phosphatidate in 2 mL chloroform:MeOH:water 63.5:31.5:5, add 500 μL 100 mM HCl, mix rapidly. Remove the lower phase and evaporate it to dryness, reconstitute the residue in 500 μL diethyl ether, add 1 mL diazomethane in diethyl ether, evaporate to dryness under a stream of nitrogen, dissolve the residue in 20 μL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrosorb RP-18
Mobile phase: MeCN:MeOH:isopropanol:water 50:18:27:5

Flow rate: 1.5
Detector: UV 205

CHROMATOGRAM
Retention time: 9.4-36.0 (depending on structure)

KEY WORDS
derivatization

REFERENCE
Nakagawa,Y.; Waku,K. Improved procedure for the separation of the molecular species of dimethylphosphatidate by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *381*, 225–231.

SAMPLE
Matrix: formulations
Sample preparation: Dilute liposome dispersions 10-fold with chloroform:MeOH 60:40, centrifuge at 2700 g for 15 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax amino
Mobile phase: MeCN:MeOH:buffer 64:28:8 (Buffer was 10 mM phosphoric acid adjusted to pH 4.8 with dilute ammonium hydroxide solution. To prepare mobile phase mix MeCN and MeOH and then add buffer.)
Flow rate: 1.5
Injection volume: 5-20
Detector: RI

CHROMATOGRAM
Retention time: 5
Limit of detection: 22 μg/mL

OTHER SUBSTANCES
Simultaneous: acyl lysophosphatidylcholine, acyl lysophosphatidylglycerol, phosphatidylglycerol

KEY WORDS
liposome dispersions

REFERENCE
Grit,M.; Crommelin,D.J.A.; Lang,J. Determination of phosphatidylcholine, phosphatidylglycerol and their lyso forms from liposome dispersions by high-performance liquid chromatography using high-sensitivity refractive index detection, *J.Chromatogr.*, **1991**, *585*, 239–246.

SAMPLE
Matrix: lung lavage fluid
Sample preparation: Centrifuge lung lavage fluid at 4° at 450 g for 10 min. Shake 10 mL supernatant and 40 mL chloroform:MeOH 2:1 at 4° for 3 min. Remove the lower organic phase and wash it with 2 mL 50 mM NaCl, centrifuge, dry under a stream of nitrogen at 45°, reconstitute with 500 μL mobile phase, vortex at 4° for 1 min, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2.1 5 μm Encapharm 100 spherical silica gel (Molnar, Berlin)
Column: 120 × 4.6 5 μm Encapharm 100 spherical silica gel (Molnar, Berlin)
Mobile phase: Gradient. A was chloroform:MeOH:ammonium hydroxide 80:19.5:0.5. B was chloroform:MeOH:water:ammonium hydroxide 60:34:5.5:0.5. A:B from 100:0 to 0:100 over 14 min, return to initial conditions over 7 min, re-equilibrate for 10 min.
Column temperature: 30
Flow rate: 1
Injection volume: 100
Detector: evaporative light-scattering, SEDERE Sedex-45, evaporation temperature 50°, nebulization gas nitrogen, pressure 200 kPa, flow 6 L/min, response is non-linear but proportional to the power 1.7 of the mass and must be calibrated for each compound

CHROMATOGRAM
Retention time: 13.11
Limit of detection: 40 ng

OTHER SUBSTANCES
Extracted: diarachidoylphosphatidylcholine, dilinoleylphosphatidylcholine, dipalmitoylphosphatidylcholine, diphosphatidylglycerol, lysophosphatidylcholine, phosphatidic acid, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylserine, sphingomyelin

KEY WORDS
normal phase

REFERENCE
Bünger,H.; Pison,U. Quantitative analysis of pulmonary surfactant phospholipids by high-performance liquid chromatography and light-scattering detection, *J.Chromatogr.B*, **1995**, *672*, 25–31.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 50 × 5 5 μm Hypersil silica
Column: 250 × 5 5 μm Hypersil silica
Mobile phase: MeCN:MeOH:water 50:45:6.5
Flow rate: 1
Detector: RI

CHROMATOGRAM
Retention time: 13

OTHER SUBSTANCES
Simultaneous: degradation products, free fatty acids, lysophosphatidylcholine

KEY WORDS
eggs

REFERENCE
Christie,W.W.; Hunter,M.L. High-performance liquid chromatography in the analysis of the products of phospholipase A hydrolysis of phosphatidylcholine, *J.Chromatogr.*, **1984**, *294*, 489–493.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 3 μm Spherisorb silica
Mobile phase: Gradient. Isopropanol:hexane:water from 58:40:2 to 52:40:8 over 7 min, maintain at 52:40:8 for 8 min.
Flow rate: 1.25
Injection volume: 20
Detector: evaporative light-scattering detector

CHROMATOGRAM
Retention time: 11.5

OTHER SUBSTANCES
Simultaneous: cholesterol, palmitic acid, phosphatidylethanolamine, phosphatidylserine, sphingomyelin

KEY WORDS
normal phase

REFERENCE
Supelco Catalog, **1993**, p. 760.

SAMPLE
Matrix: solutions
Sample preparation: Inject 100 mg soybean oil as a solution in hexane.

HPLC VARIABLES
Column: 250 × 4.6 15-40 μm silica gel 60
Mobile phase: Gradient. Hexane:isopropanol:water 55:44:4 for 22 min, 55:44:5.7 for 8 min, 55:44:7 for 70 min (step gradient).
Flow rate: 1
Detector: UV 214

CHROMATOGRAM
Retention time: 50

OTHER SUBSTANCES
Simultaneous: lysophosphatidylcholine, phosphatidic acid, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine

KEY WORDS
semi-preparative; also details of a preparative procedure; normal phase

REFERENCE
De Meulenaer,B.; Van der Meeren,P.; Vanderdeelen,J.; Baert,L. Optimization of a chromatographic method for the gram-scale preparative fractionation of soybean phospholipids, *Chromatographia*, **1995**, *41*, 527–531.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 50 μL aliquot of a solution in chloroform:MeOH 98:2 on to column A and column B in series and elute with mobile phase A, after 10 min elute with mobile phase B, after 10 min remove column A from the circuit, monitor the effluent from column B, after 30 min elute column A with mobile phase B and monitor the effluent from column A.

HPLC VARIABLES
Column: A 250 × 4 5 μm LiChrosorb diol; B 250 × 4 5 μm LiChrospher Si100
Mobile phase: A MeCN; B MeCN:MeOH:phosphoric acid 93:5:1.5
Flow rate: 1
Injection volume: 50
Detector: UV 205

CHROMATOGRAM
Retention time: 46

OTHER SUBSTANCES
Simultaneous: lysophosphatidylcholine, lysophosphatidylethanolamine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, sphingomyelin

KEY WORDS
column-switching

REFERENCE
Soudant,P.; Marty,Y.; Moal,J.; Samain,J.F. Separation of major polar lipids in Pecten maximus by high-performance liquid chromatography and subsequent determination of their fatty acids using gas chromatography, *J.Chromatogr.B*, **1995**, *673*, 15–26.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 30 mg/mL solution of lyophilized lipids in MeOH, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μPorosil (Waters)
Mobile phase: MeCN:MeOH:water 25:70:5
Flow rate: 2
Injection volume: 20
Detector: UV 205

CHROMATOGRAM
Retention time: 3.23

OTHER SUBSTANCES
Simultaneous: cardiolipin, cholesterol

KEY WORDS
egg

REFERENCE
Choudhari,K.B.; Jayanthi,S.; Murty,R.B.; Matharu,R.P. A high-performance liquid chromatographic method for the analysis of lipids from lyophilized formulations, *J.Chromatogr.A*, **1996**, *724*, 343–347.

SAMPLE
Matrix: tissue
Sample preparation: Blend squid skin, add 5 volumes acetone, agitate at 25° for 2 h, filter, wash the solids three times with one volume of cold acetone. Keep the filtrate at -20° for 16 h, centrifuge at 2500 rpm for 10 min, discard the acetone supernatant, dry the pellet under a stream of nitrogen. Dissolve in the initial mobile phase, inject an aliquot. (All solvents contain 0.1% BHT.)

HPLC VARIABLES
Column: 250 × 7.2 5μm Lichrosorb Si 60
Mobile phase: Gradient. A:B:C from 42:52:6 to 32:52:16 over 20 min. A was hexane; B was isopropanol:chloroform (80:20); C was isopropanol:water (50:50).
Flow rate: 2.5
Injection volume: 250
Detector: ELSD (Cunow, France)

OTHER SUBSTANCES
Extracted: phosphatidylethanolamine, phosphatidylinotisol, phosphatidylserine, sphingomyelin

KEY WORDS
skin; squid; normal phase

REFERENCE
Baudimant,G.; Maurice,M.; Landrein,A.; Durand,G.; Durand,P. Purification of phosphatidylcholine with high content of DHA from squid Illex argentinus by countercurrent chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1793–1804.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Potter-Elvehjem) liver or lung with 20 volumes chloroform: MeOH 2:1, filter (paper), wash with a volume of 50 mM NaCl equal to one-fifth the volume of extract, centrifuge (J.Biol.Chem. 1957, 226, 497). Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in chloroform, add 100 μL 1 mM IS in chloroform. Purify by normal phase HPLC using hexane:isopropanol:water 42:56:6.3 at 1 mL/min on a 300 × 4.9 10 μm μPorasil column using UV 200 detection, collect fraction eluting between 26 and 32 min, evaporate, dissolve in the minimum amount of trifluoroethanol, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Apex ODS 2
Mobile phase: MeOH:water 92.5:7.5 containing 40 mM choline chloride

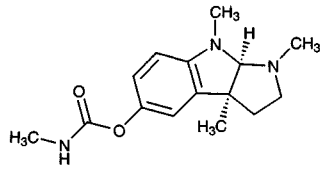
Column temperature: 50
Flow rate: 1
Detector: F ex 340 em 460 following post-column derivatization. The column effluent was mixed with the reagent pumped at 3 mL/min in a 15 μL mixing chamber and the mixture flowed through a 3 m × 0.5 mm i.d. PTFE coil at 50° to the detector. Reagent was water containing 150 μL/L 3 mM 1,6-diphenyl-1,3,5-hexatriene in THF:Tween 20 99.999:0.001

CHROMATOGRAM
Retention time: 35-57
Internal standard: phosphatidylcholine 14:0/14:0 (20)

KEY WORDS
rat; liver; lung; post-column reaction

REFERENCE
Postle,A.D. Method for the sensitive analysis of individual molecular species of phosphatidylcholine by high-performance liquid chromatography using post-column fluorescence detection, *J.Chromatogr.*, **1987**, *415*, 241–251.

Physostigmine



Molecular formula: $C_{15}H_{21}N_3O_2$
Molecular weight: 275.35
CAS Registry No.: 57-47-6, 57-64-7 (salicylate), 64-47-1 (sulfate)
Merck Index: 7540
Lednicer No.: 1 111

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 μL 1 mg/mL neostigmine + 20 μL 1 μg/mL dimethylphysostigmine + 500 μL 100 mM pH 7 picric acid (Caution! Do not allow to dry! Dry picric acid is explosive!) + 500 μL 100 mM NaH_2PO_4, vortex thoroughly, add 10 mL water-saturated dichloromethane, mix vigorously by inverting and vortexing for 15 s, centrifuge at 1000 g for 10 min. Discard the upper aqueous phase and add 2 mL water-saturated dichloromethane, mix vigorously by inverting and vortexing for 15 s, centrifuge at 1000 g for 7 min. Remove the organic phase and add it to 200 μL 1 mM pH 1.8 tetrabutylammonium hydrogen sulfate, mix vigorously by inverting and vortexing for 15 s, centrifuge at 1000 g for 7 min, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: 5 × 3.2 7 μm silica
Column: 250 × 4.6 5 μm Ultrasphere-Si
Mobile phase: MeCN:buffer 20:80 (Buffer was 10 mM NaH_2PO_4 containing 2.5 mM tetramethylammonium chloride, pH 3.0.)
Flow rate: 1
Injection volume: 50
Detector: F ex 240 em 360

CHROMATOGRAM
Retention time: 7.08
Internal standard: dimethylphysostigmine (9.63)
Limit of detection: 0.1 ng/mL
Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: neostigmine

KEY WORDS
plasma

REFERENCE
Elsayed,N.M.; Ryabik,J.R.G.; Ferraris,S.; Wheeler,C.R.; Korte,D.W.,Jr. Determination of physostigmine in plasma by high-performance liquid chromatography and fluorescence detection, *Anal.Biochem.*, **1989**, *177*, 207–211.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 50 μL 50 μg/mL neostigmine bromide in water, filter (Amicon Centricon, 10000 molecular mass cut-off) while centrifuging at 4° at 7000 g for 70 min, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES
Guard column: C18 (Waters)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:buffer 40:60, pH adjusted to 2.1 (Buffer was 5 mM NaH_2PO_4 containing 1% acetic acid and 0.5 mM 1-octanesulfonic acid.)
Flow rate: 1.5
Injection volume: 100
Detector: radioactivity (Radiomatic Instruments Flo-One B) (The column effluent mixed with Ultrafluor (National Diagnostics) pumped at 4 mL/min and passed to the flow cell.)

CHROMATOGRAM
Retention time: 10.1
Limit of detection: 0.05 ng/mL

OTHER SUBSTANCES
Extracted: eseroline

KEY WORDS
tritium labeled; guinea pig; plasma; pharmacokinetics

REFERENCE
Lukey,B.J.; Marlow,D.D.; Clark,C.R.; McCluskey,M.P.; Lieske,C.N. Application of a new radiometric high-performance liquid chromatographic assay to define physostigmine pharmacokinetics in guinea pigs, *J.Chromatogr.*, **1989**, *493*, 117–124.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL 200 mg octadecyl SPE cartridge with 2 column volumes of MeOH and 2 column volumes of 100 mM pH 4 phosphate buffer. 1 mL Serum + 5 μL 1 mg/mL neostigmine bromide in 100 mM pH 4 NaH_2PO_4, vortex for 15 s, add 1 mL 1 mM reagent in 100 mM pH 4 phosphate buffer, mix for 30 s, add to the SPE cartridge, wash with 4 mL water, elute with 200 μL MeOH:water 95:5. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot. (Synthesize reagent, sodium α-(3,4-dimethoxyphenyl) cinnamonitrile-2'-sulfonate, as follows. Add 5 mL 10% KOH in water to a stirred solution of 20 mmoles 3,4-(dimethoxyphenyl)acetonitrile and 20 mmoles 2-formylbenzenesulfonic acid, sodium salt hydrate (sodium benzaldehyde-2-sulfonate) in 50 mL EtOH at 50°, stir at 50° for 5 min, cool (evaporate to near dryness, if necessary), filter to obtain sodium α-(3,4-dimethoxyphenyl) cinnamonitrile-2'-sulfonate (mp of p-toluidine salt is 218-223°) (J. Chem. Eng. Data 1975, 20, 215).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm diol (ES Industries, Marlton NJ)
Mobile phase: MeOH:50 mM pH 4 NaH_2PO_4 20:80 containing 500 μM sodium α-(3,4-dimethoxyphenyl) cinnamonitrile-2'-sulfonate
Flow rate: 1
Injection volume: 50

Detector: F ex 243 em 418 (cutoff filter) following post-column extraction. The column effluent mixed with dichloromethane pumped at 1 mL/min and the mixture flowed through a 90 cm × 0.3 mm ID knitted PTFE coil to a 50 μL membrane phase separator using a polyethylene-backed 0.5 μm Fluoropore membrane filter (design in paper). The organic phase flowed to the detector.

CHROMATOGRAM
Retention time: 6.42
Internal standard: neostigmine (11.16)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, eseroline
Also analyzed: amantadine, amphetamine, atropine, chlorpheniramine, clidinium bromide, N,N-dimethyl-N-benzyltetradecylammonium chloride, guanethidine, hydralazine, imipramine, malachite green, promazine, propantheline bromide
Noninterfering: chlordiazepoxide

KEY WORDS
post-column extraction; SPE; serum; silanize glassware; post-column reaction

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REFERENCE
Quinn,K.D.; Stewart,J.T. A high performance liquid chromatographic post-column fluorescent ion pair extraction system: application to physostigmine and its metabolite eseroline in human serum, *Biomed.Chromatogr.*, **1991**, *5*, 8–13.

SAMPLE
Matrix: blood
Sample preparation: Add physostigmine octylcarbamate to freshly-drawn blood in a final concentration of 500 nM, prepare plasma, add IS, purify by SPE, inject an aliquot. (Keep samples on ice throughout procedure.)

HPLC VARIABLES
Column: two 200 mm (?) long narrow-bore normal phase columns in series (Brownlee)
Mobile phase: MeCN:10 mM formic acid:50 mM Tris buffer 27:52:21
Flow rate: 0.15
Detector: F ex 250 em 345

CHROMATOGRAM
Internal standard: N-methylphysostigmine
Limit of detection: 0.055 ng/mL

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Asthana,S.; Greig,N.H.; Hegedus,L.; Holloway,H.H.; Raffaele,K.C.; Schapiro,M.B.; Soncrant,T.T. Clinical pharmacokinetics of physostigmine in patients with Alzheimer's disease, *Clin.Pharmacol.Ther.*, **1995**, *58*, 299–309.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 500 mM sodium bicarbonate + 5 mL n-hexane, shake for 10 min, centrifuge at 1500 g for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL MeCN:MeOH 50:50, inject a 70 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm silica (Violet)
Mobile phase: MeCN:MeOH:80 mM ammonium nitrate 50:40:10, pH 8.90
Flow rate: 1

Injection volume: 70
Detector: E, glassy carbon electrode +0.75 V

CHROMATOGRAM
Retention time: 6
Internal standard: physostigmine

OTHER SUBSTANCES
Extracted: eptastigmine

KEY WORDS
plasma; physostigmine is IS

REFERENCE
Imbimbo,B.P.; Licini,M.; Schettino,M.; Mosca,A.; Onelli,E.; Zecca,L.; Giustina,A. Relationship between pharmacokinetics and pharmacodynamics of eptastigmine in young healthy volunteers, *J.Clin.Pharmacol.*, **1995**, *35*, 285–290.

SAMPLE
Matrix: diffusate, tissue
Sample preparation: Homogenize (Polytron PCU-2) 150-200 mg skin and diazepam with 4 mL chloroform, repeat homogenization, filter (phase-separating paper) extracts. Make the residue alkaline with 2 mL 10% NaOH, extract twice with 4 mL portions of chloroform, wash the extracts twice with 2 mL portions of water, filter (phase-separating paper) the organic layer. Combine all the chloroform layers and evaporate them to dryness under a stream of air, reconstitute the residue in 1 mL mobile phase, filter (microfilter), inject an aliquot.

HPLC VARIABLES
Guard column: 20 × 4 40 μm ODS (Valco)
Column: 150 × 4.6 5 μm Spherisorb ODS-I
Mobile phase: MeCN:water 52:48 containing 10 mM octanesulfonic acid and 1% acetic acid, pH 3.5
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 4.5
Internal standard: diazepam (6.0)
Limit of detection: 5 μg/g

OTHER SUBSTANCES
Extracted: tacrine

KEY WORDS
skin; pharmacokinetics; stability-indicating

REFERENCE
Lau,S.W.J.; Chow,D.; Feldman,S. Simultaneous determination of physostigmine and tetrahydroaminoacridine in a transdermal permeation study by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *526*, 87–95.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.2

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

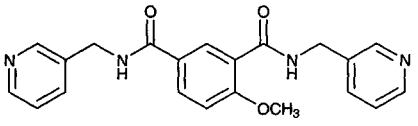
HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 35:1.5:0.5:63
Flow rate: 1.5
Injection volume: 10
Detector: UV 261

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: salicylic acid

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

Picotamide



Molecular formula: $C_{21}H_{20}N_4O_3$
Molecular weight: 376.41
CAS Registry No.: 32828-81-2
Merck Index: 7560

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma + 20 μL 0.05 mg/mL bamifylline in MeOH + 60 μL 25% aqueous ammonia, mix, add 5 mL chloroform:isopropanol 95:5, shake for 20 min, centrifuge at 2000 g for 15 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL 0.6 M HCl, add 600 μL ethyl acetate, vortex for 15 s, centrifuge at 2000 g for 3 min. Remove the acidic aqueous phase and evaporate it to dryness under vacuum, reconstitute the residue in 100 μL MeOH:water 50:50, inject a 25 μL aliquot. Urine. 0.1-1 mL Urine + 20 μL 2.5 mg/mL bamifylline in MeOH + 100 μL 25% aqueous ammonia, mix, add 5 mL chloroform:isopropanol 95:5, shake for 20 min, centrifuge at 2000 g for 15 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL 1.2 M HCl, add 600 μL ethyl acetate, vortex for 15 s, centrifuge at 2000 g for 3 min. Remove the acidic aqueous phase and evaporate it to dryness under vacuum, reconstitute the residue in 100 μL MeOH: water 50:50, inject a 25 μL aliquot.

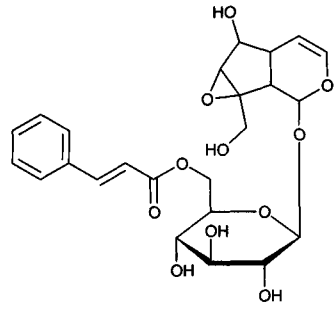
HPLC VARIABLES
Column: 250 × 4 5 μm LiChrosorb RP-Select B C8
Mobile phase: MeCN:50 mM pH 5.5 NaH_2PO_4 28:72
Flow rate: 1
Injection volume: 25
Detector: UV 230

CHROMATOGRAM
Retention time: 10
Internal standard: bamifylline (7)
Limit of quantitation: 1 μg/mL (urine), 5 ng/mL (plasma)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Fossati,T.; Parisi,S.; Abbiati,G.; Castiglioni,C. Determination of picotamide in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *577*, 382–386.

Picroside



Molecular formula: $C_{24}H_{28}O_{11}$
Molecular weight: 492.47
CAS Registry No.: 27409-30-9

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 500 μL MeOH, vortex for 2 min, centrifuge at 3000 rpm for 10 min. Transfer the supernatant to another tube and evaporate the MeOH under a stream of nitrogen, extract 3 times with 500 μL portions of ethyl acetate. Evaporate the combined ethyl acetate layers to dryness. Reconstitute the residue with 50 μL MeOH, inject a 20 μL aliquot.

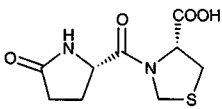
HPLC VARIABLES
Guard column: 4 × 4 5 μm C18
Column: 250 × 4 5 μm C18
Mobile phase: MeCN:100 mM acetic acid 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 8.5
Limit of quantitation: 100 ng/ mL

KEY WORDS
plasma; rabbit

REFERENCE
Dwivedi,A.K.; Kulkarni,D.; Singh,S. Sensitive high-performance liquid chromatographic assay method for the determination of picroside I in plasma, *J.Chromatogr.B*, **1997**, *698*, 317–320.

Pidotimod



Molecular formula: $C_9H_{12}N_2O_4S$
Molecular weight: 244.27
CAS Registry No.: 121808-62-6
Merck Index: 7574

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 μL 400 μg/mL oxiracetam + 100 μL 35% perchloric acid, vortex for 15 s, sonicate for 10 min, centrifuge at 12000 rpm for 10 min. Remove a 500 μL aliquot of the supernatant and add it to 500 μL mobile phase, vortex for 15 s, centrifuge at 12000 rpm for 10 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: 15 × 3.2 PRP (Brownlee)

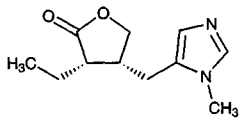
Column: 300 × 7.8 Aminex Ion-Exclusion HPX 874 (Bio-Rad)
Mobile phase: MeCN:0.05% sulfuric acid 12:88
Flow rate: 0.6
Injection volume: 40
Detector: UV 210

CHROMATOGRAM
Retention time: 16.5
Internal standard: oxiracetam (13.8)
Limit of detection: 100 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Dal Bo,L.; Broccali,G.P.; Silingardi,S.; Coppi,G. A new HPLC method for pidotimod plasma levels determination, *Boll.Chim.Farm.*, **1993**, *132*, 126–128.

Pilocarpine



Molecular formula: $C_{11}H_{16}N_2O_2$
Molecular weight: 208.26
CAS Registry No.: 92-13-7, 54-71-7 (HCl), 148-72-1 (nitrate)
Merck Index: 7578

SAMPLE
Matrix: aqueous humor
Sample preparation: 100 μL Aqueous humor + 500 μL 300 mM pH 8.4 potassium bicarbonate + 1 mL dichloromethane, vortex for 1 min, centrifuge at 2000 rpm for 5 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL 250 μg/mL p-nitrobenzyl bromide in MeCN, heat in a sealed tube at 40° for 24 h, cool, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water 80:20 containing 1 mM sodium octanesulfonate
Flow rate: 1.6
Detector: UV 254

CHROMATOGRAM
Retention time: 11
Limit of detection: <50 ng/mL

OTHER SUBSTANCES
Simultaneous: isopilocarpine

KEY WORDS
derivatization; rabbit; silanize glassware with dimethyldichlorosilane

REFERENCE
Mitra,A.K.; Baustian,C.L.; Mikkelson,T.J. High-performance liquid chromatographic determination of pilocarpine in aqueous humor: derivatization by quaternization of methylimidazole tertiary amine group, *J.Pharm.Sci.*, **1980**, *69*, 257–261.

SAMPLE
Matrix: aqueous humor

Sample preparation: 200 μL Aqueous humor + 400 μL MeOH, centrifuge at 8000 g for 5 min. Remove the supernatant and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 75 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Cosmosil 5Ph (Nacalai Tesque)
Mobile phase: MeCN:0.1% acetic acid 5:95
Column temperature: 50
Flow rate: 1
Injection volume: 75
Detector: MS, Hitachi Model M-80B double-focusing, M-8093 APCI interface, nebulizer 350°, vaporizer 390°, drift voltage 140 V, corona discharge 12 μA, full scan m/z 1-600 in 4 s, SIM m/z 209 (Divert mobile phase to waste until pilocarpine elutes.)

CHROMATOGRAM
Retention time: 5.1
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: degradation products, isopilocarpic acid, isopilocarpine, pilocarpic acid

KEY WORDS
rabbit; pharmacokinetics

REFERENCE
Matsuura,K.; Kuwano,M.; Takashima,H. Determination of pilocarpine in aqueous humour by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry, *J.Chromatogr.*, **1993**, *621*, 173–180.

SAMPLE
Matrix: blood
Sample preparation: Add 125 mg NaF to each 1 mL of blood collected. 500 μL Plasma + 500 μL clonidine in water + 3 mL dichloromethane, shake gently for 10 min, centrifuge at 2100 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 150 μL 1 mM HCl, vortex for 4 min, sonicate for 45 s, add 2 mL diethyl ether, vortex for 2 min, centrifuge at 2100 g for 5 min, discard the ether phase, apply a vacuum to the aqueous phase for 10 s, inject a 35-100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: 15 × 4.6 37 μm Corasil C18
Column: 150 × 4.6 5 μm Spherisorb ODS-1
Mobile phase: MeCN:MeOH:7 mM pH 4.0 potassium phosphate buffer 30:15:55
Flow rate: 1.2
Injection volume: 35-100
Detector: UV 214

CHROMATOGRAM
Retention time: 7.2
Internal standard: clonidine (12)
Limit of detection: 5 ng/mL
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: pentobarbital

KEY WORDS
plasma; human; dog

REFERENCE
Weaver,M.L.; Tanzer,J.M.; Kramer,P.A. High-performance liquid chromatographic determination of pilocarpine in plasma, *J.Chromatogr.*, **1992**, *581*, 293–296.

SAMPLE

Matrix: blood, saliva, urine

Sample preparation: Mix 50 μL urine with 5 μL 10% sodium bicarbonate solution add 50 μL 500 ng/mL pilosine in MeOH. Mix 1.5 mL saliva with 90 μL 10% sodium bicarbonate, add 50 μL 500 ng/mL pilosine in MeOH. Mix 3 mL Plasma with 50 μL 500 ng/mL pilosine in MeOH. Extract these samples twice with 3 mL portions of chloroform. Combine the organic layers and evaporate them to dryness, reconstitute the residue in 200 μL 0.08% 4-bromomethyl-7-methoxycoumarin in acetone, heat at 37° for 48 h, evaporate to dryness, reconstitute with 1 mL mobile phase, centrifuge at 20000 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Guard column: 20 × 4.6 cyanopropyl silica (Brownlee)
Column: 220 × 4.6 cyanopropyl silica (Brownlee)
Mobile phase: MeCN:buffer 30:70 (Buffer was 3 mM diethylamine adjusted to pH 3.5 with 1 M phosphoric acid.)
Column temperature: 37
Flow rate: 1
Injection volume: 100
Detector: F ex 324 em 400

CHROMATOGRAM

Retention time: 13.86
Internal standard: pilosine (12.36)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Interfering: isopilocarpine

KEY WORDS

derivatization; silanize all glassware with dichlorodimethylsilane for 12 h; plasma; pharmacokinetics

REFERENCE

Aromdee,C.; Fawcett,J.P.; Ledger,R. Sensitive high-performance liquid chromatographic assay for pilocarpine in biological fluids using fluorescence derivatization, *J.Chromatogr.B*, **1996**, *677*, 313–318.

SAMPLE

Matrix: blood, urine

Sample preparation: Blood. 10 mL Whole blood + 250 μL 5% disodium EDTA, vortex, centrifuge at 1500 g for 10 min. Remove a 4 mL aliquot of the plasma and add it to 125 μL 5% disodium EDTA, mix. Remove a 1 mL aliquot and filter (Amicon Centrifree with 30000 Da cut-off), while centrifuging at 2000 g for 15 min, inject a 25 μL aliquot of the ultrafiltrate. Urine. Adjust urine to pH 5 with 100 mM HCl, dilute 20-fold with 200 mM pH 3.7 sodium acetate buffer, inject a 25 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Inertsil ODS
Mobile phase: MeCN:50 mM ammonium acetate buffer adjusted to pH 4.0 with trifluoroacetic acid 3:97
Flow rate: 1
Injection volume: 25
Detector: MS, PE Sciex API III plus, positive ion mode, multiple monitoring mode, APCI, nebulizer 500 °, m/z 209

CHROMATOGRAM

Retention time: 18
Limit of detection: 500 pg/mL (plasma), 10 ng/mL (urine)
Limit of quantitation: 2 ng/mL (plasma), 40 ng/mL (urine)

OTHER SUBSTANCES

Extracted: degradation products

KEY WORDS
plasma; ultrafiltrate; whole blood

REFERENCE
Van de Merbel,N.C.; Tinke,A.P.; Oosterhuis,B.; Jonkman,J.H.G.; Bohle,J.F. Determination of pilocarpine, isopilocarpine, pilocarpic acid and isopilocarpic acid in human plasma and urine by high-performance liquid chromatography with tandem mass spectrometric detection, *J.Chromatogr.B*, **1998**, *708*, 103–112.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 214.6

CHROMATOGRAM
Retention time: 4.622

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve ophthalmic gel in mobile phase so that the pilocarpine concentration is 40 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Cyclobond β-cyclodextrin (Rainin)
Mobile phase: Water containing 40 g/L ammonium sulfate and 20 mL/L triethylamine, pH adjusted to 4.0 with phosphoric acid.
Flow rate: 1
Injection volume: 10
Detector: UV 214

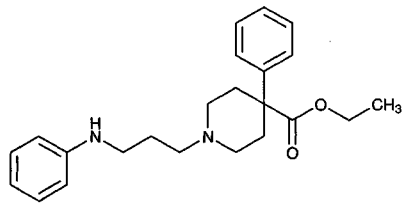
CHROMATOGRAM
Retention time: 7.5
Limit of detection: 0.2-0.3% (of pilocarpine present)

OTHER SUBSTANCES
Simultaneous: degradation products, isopilocarpic acid, isopilocarpine, pilocarpic acid

KEY WORDS
ophthalmic gel

REFERENCE
Sternitzke,K.D.; Fan,T.Y.; Dunn,D.L. High-performance liquid chromatographic determination of pilocarpine hydrochloride and its degradation products using a β-cyclodextrin column, *J.Chromatogr.*, **1992**, *589*, 159–164.

Piminodine



Molecular formula: $C_{23}H_{30}N_2O_2$
Molecular weight: 366.50
CAS Registry No.: 13495-09-5, 7081-52-9 (ethanesulfonate)
Merck Index: 7587
Lednicer No.: 1 301

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.8

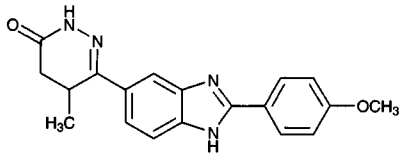
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine,

phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Pimobendan



Molecular formula: $C_{19}H_{18}N_4O_2$
Molecular weight: 334.38
CAS Registry No.: 118428-36-7, 74150-27-9
Merck Index: 7588
Lednicer No.: 5 117

SAMPLE

Matrix: blood
Sample preparation: Extract from plasma using SPE.

HPLC VARIABLES

Column: 5 μm ODS-Hypersil
Mobile phase: MeOH:water 59:46 containing 2.5 g/L ammonium acetate
Detector: F ex 332 em 405 following post-column reaction. The column effluent mixed with MeOH:85% orthophosphoric acid:water 60:20:20 pumped at 0.2 mL/min and the mixture flowed to the detector.

CHROMATOGRAM

Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

pig; plasma; SPE; post-column reaction

REFERENCE

Verdouw,P.D.; Hartog,J.M.; Duncker,D.J.; Roth,W.; Saxena,P.R. Cardiovascular profile of pimobendan, a benzimidazole-pyridazinone derivative with vasodilating and inotropic properties, *Eur.J.Pharmacol.*, **1986**, *126*, 21–30.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 100 μL 10 μg/mL carbamazepine in n-propanol + 5 mL MeCN, rotate for 10 min, centrifuge at 1880 g for 10 min. Remove the supernatant and evaporate it to 0.8-1 mL under a stream of air, add 500 μL 20 mM pH 5 ammonium hydrogen phosphate buffer, add 5 mL dichloromethane:n-propanol 70:30, vortex for 30 s, centrifuge at 1880 g for 5 min. Remove the organic layer and evaporate it to dryness, add 500 μL water and

3 mL diethyl ether to the residue, vortex for 30 s. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 350 μL mobile phase, inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 5 μm Spheri-5 silica
Column: 250 × 4.6 5 μm Spheri-5 silica + 250 × 4.6 10 μm Chiralcel OD in series
Mobile phase: n-Hexane:EtOH:diethylamine 75:25:0.1
Column temperature: 35
Flow rate: 1
Injection volume: 200
Detector: UV 328

CHROMATOGRAM
Retention time: 17.3 (+), 20.9 (-)
Internal standard: carbamazepine (13.7)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; chiral; pharmacokinetics

REFERENCE
Chu,K.-M.; Shieh,S.-M.; Wu,S.H.; Hu,O.Y.-P. Enantiomeric separation of a cardiotonic agent pimobendan and its major active metabolite, UD-CG 212 BS, by coupled achiral-chiral normal-phase high-performance liquid chromatography, *J.Chromatogr.Sci.*, **1992**, *30*, 171–176.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond Elut PH SPE cartridge with 3 mL MeOH and 3 mL water. 100 μL Plasma + 1 mL 100 mM pH 9.5 phosphate buffer, add to the SPE cartridge, wash with 3 mL water, dry by pulling air through the cartridge for 15 min, elute with 1 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL EtOH:n-hexane 50:50, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Sumchiral OA-4400 (Sumika)
Mobile phase: n-Hexane:EtOH:acetic acid 300:120:1
Column temperature: 40
Flow rate: 0.8
Injection volume: 100
Detector: F ex 330 em 415 following post-column reaction. The column effluent mixed with EtOH:acetic acid pumped at 0.3 mL/min and the mixture flowed to the detector.

CHROMATOGRAM
Retention time: 14.4 (-), 15.1 (+)
Limit of detection: 1.25 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
chiral; post-column reaction; plasma; rat; SPE; pharmacokinetics

REFERENCE
Asakura,M.; Nagakura,A.; Tarui,S.; Matsumura,R. Simultaneous determination of the enantiomers of pimobendan and its main metabolite in rat plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *614*, 135–141.

SAMPLE
Matrix: cell incubations
Sample preparation: Inject 200 μL cell incubation on to column A with mobile phase A then switch to mobile phase B (start the gradient) and elute to waste, after 4 min direct the effluent from column A on to column B, after 30 min remove column A from the circuit.

HPLC VARIABLES
Column: A 40 × 4.6 37-75 μm Porasil B; B 30 mm long 5 μm Hypersil ODS + 125 × 4.6 5 μm Hypersil ODS
Mobile phase: A 1% pH 6.8 ammonium acetate buffer; B Gradient. A was water:25% ammonia 100:0.02. B was MeOH. A:B 100:0 for 6.9 min, to 95:5 over 0.1 min, to 85:15 over 11 min, to 0:100 over 9 min, maintain at 0:100 for 3 min, re-equilibrate at initial conditions for 2 min.
Column temperature: 28
Flow rate: 1
Injection volume: 200
Detector: F ex 332 em 405 following post-column reaction. The column effluent mixed with MeOH:water:85% orthophosphoric acid 60:20:20 pumped at 0.2 mL/min and flowed to the detector.

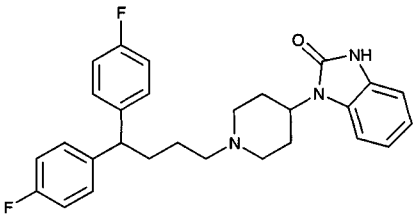
CHROMATOGRAM
Retention time: 28

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver; hepatocytes; post-column reaction; column-switching

REFERENCE
Pahernik,S.A.; Schmid,J.; Sauter,T.; Schildberg,F.W.; Koebe,H.G. Metabolism of pimobendan in long-term human hepatocyte culture: in vivo-in vitro comparison, *Xenobiotica*, **1995**, *25*, 811–823.

Pimozide



Molecular formula: $C_{28}H_{29}F_2N_3O$
Molecular weight: 461.55
CAS Registry No.: 2062-78-4
Merck Index: 7589
Lednicer No.: 2 290

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL whole blood with 500 ng IS, add 500 μL 2% pH 9.5 sodium tetraborate and 8 mL n-butanol:hexane 5:95. Extract for 30 min, centrifuge. Remove the organic layer and add it to 200 μL 0.2% orthophosphoric acid. Extract for 30 min, inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 150 × 3.9 5 μm NovaPak-Phenyl
Mobile phase: MeCN:10 mM KH_2PO_4 55:45, adjusted to pH 3.0
Flow rate: 1.5
Injection volume: 30
Detector: UV 214

CHROMATOGRAM
Internal standard: pentazocine
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: sertraline

REFERENCE
McIntyre,I.M.; King,C.V.; Staikos,V.; Gall,J.; Drummer,O.H. A fatality involving moclobemide, sertraline, and pimozide, *J.Forensic Sci.*, **1997**, *42*, 951–953.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 200 ng/mL 3-methylpimozide in 1% phosphoric acid + 1 mL 1 M NaOH + 5 mL n-hexane:isoamyl alcohol 98:2, shake reciprocally for 10 min, centrifuge at 1900 g for 5 min. Remove the organic layer and ad it to 100-200 μL 100 mM phosphoric acid, shake, centrifuge, discard the organic layer, inject almost all of the aqueous layer.

HPLC VARIABLES
Guard column: 10 × 4 5 μm TSK-GEL LS-410 (Toyo Soda)
Column: 150 × 4 5 μm TSK-GEL LS-410 (Toyo Soda)
Mobile phase: MeCN:buffer 48:52 (Buffer was 20 mM KH_2PO_4 adjusted to pH 2.5 with 20 mM phosphoric acid.)
Flow rate: 1
Injection volume: 100-200
Detector: F ex 210 em >320 or UV 280

CHROMATOGRAM
Retention time: 6
Internal standard: 3-methylpimozide (8)
Limit of detection: 0.3 ng/mL, 5 ng/mL (UV)

OTHER SUBSTANCES
Noninterfering: haloperidol, levomepromazine, sulpride, thioridazine, thiothixene

KEY WORDS
plasma

REFERENCE
Miyao,Y.; Suzuki,A.; Noda,K.; Noguchi,H. A sensitive assay method for pimozide in human plasma by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1983**, *275*, 443–449.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 281

CHROMATOGRAM
Retention time: 8.77
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: Condition a 3 mL Bond Elut Certify SPE cartridge with 2 mL MeOH and 2 mL 100 mM pH 6.0 phosphate buffer, do not allow to dry. 1 mL Blood + 6 mL 100 mM pH 6.0 phosphate buffer, vortex, sonicate, centrifuge, add the supernatant to the SPE cartridge, wash with water, wash with 1 mM pH 3.3 acetic acid, dry by suction, wash with 2 mL acetone: chloroform 50:50, elute with 3 mL ethyl acetate:ammonia 98:2. Evaporate the eluate under a stream of nitrogen at 40°, reconstitute the residue in 50 µL MeOH, inject a 10 µL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 µm Nova-Pack C18

Mobile phase: MeOH:50 mM ammonium acetate 75:25 (Mix column effluent with 50 mM ammonium acetate pumped at 0.5 mL/min.)

Flow rate: 0.6

Injection volume: 10

Detector: MS, Finnigan MAT TSQ 700 tandem quadrupole, MAT TSP-2 interface, thermospray, selective reaction monitoring m/z 462-238, collision offset -27.5 V, repeller 100 V, vaporizer 130°, source 200°, filament on 200 µA, argon 2.5 mTorr, multiplier 1500 V, dynode 15 kV, scan time 1.20 s, MSMSC factor 10

CHROMATOGRAM

Retention time: 10.20

Limit of detection: 100 pg

OTHER SUBSTANCES
Extracted: benperidol, dextromoramide, droperidol, haloperidol, methadone, penfluridol, pipamperidone, propoxyphene (dextropropoxyphene)

KEY WORDS
SPE; LC/MS

REFERENCE
Verweij,A.M.; Hordijk,M.L.; Lipman,P.J. Quantitative liquid chromatographic thermospray-tandem mass spectrometric analysis of some analgesics and tranquilizers of the methadone, butyrophenone, or diphenylbutylpiperidine groups in whole blood, *J.Anal.Toxicol.*, **1995**, *19*, 65–68.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 205.2

CHROMATOGRAM
Retention time: 17.192

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.5

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 14.36 (A), 7.96 (B)

OTHER SUBSTANCES

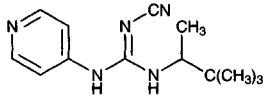
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordi-

azepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Pinacidil



Molecular formula: $C_{13}H_{19}N_5$
Molecular weight: 245.32
CAS Registry No.: 60560-33-0, 85371-64-8 (monohydrate)
Merck Index: 7592
Lednicer No.: 4 102

SAMPLE
Matrix: blood
Sample preparation: 1-2 mL Plasma + 100 µL 1 µg/mL IS + 2 mL 100 mM pH 10 carbonate buffer, extract twice with 5 mL portions of ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 250 µL MeOH:water 50:50, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 µm Lichrosorb RP18
Mobile phase: MeCN:10 mM $(NH_4)_2HPO_4$ 33:67
Flow rate: 1.2
Injection volume: 200
Detector: UV 254

CHROMATOGRAM
Retention time: 5
Internal standard: N"-cyano-N-(1-ethyl-1-methylpropyl)-N'-4-pyridylguanidine (P 1149) (6.5)

Limit of detection: 3 ng/mL

KEY WORDS
rat; dog; human; plasma; pharmacokinetics

REFERENCE
Eilertsen,E.; Hart,J.W.; Magnussen,M.P.; Sorensen,H.; Arrigoni-Martelli,E. Pharmacokinetics and distribution of the new antihypertensive agent pinacidil in rat, dog and man, *Xenobiotica*, **1982**, *12*, 177–185.

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 3 mL MeOH and 5 mL water. 1 mL Plasma + 50 μL 5 μg/mL IS in 10 mM HCl, vortex, add to the SPE cartridge, wash with 5 mL water, wash with 5 mL MeOH:water 20:80, elute with 4 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: Co-PELL ODS
Column: 250 × 4.6 6 μm Zorbax C8
Mobile phase: MeOH:buffer 45:55 (Buffer was 550 mL 100 mM sodium acetate + 1 mL morpholine, adjust pH to 4.0 with glacial acetic acid.)
Column temperature: 40
Flow rate: 1.5
Injection volume: 100
Detector: UV 284

CHROMATOGRAM
Retention time: 9
Internal standard: N'-cyano-N-(1-ethyl-1-methylpropyl)-N'-4-pyridinyl guanidine (11)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: minoxidil, triamterene
Noninterfering: allopurinol, atenolol, captopril, chlordiazepoxide, clidinium bromide, clonidine, dipyridamole, furosemide, hydralazine, hydrochlorothiazide, metoprolol, pilocarpine, propranolol, sulindac
Interfering: colchicine, prazosin

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Hamilton,M.; Farid,K.Z.; Henry,D.P. Liquid chromatographic determination of pinacidil, a new antihypertensive drug, and its major metabolite, pinacidil N-oxide, in plasma, *J.Chromatogr.*, **1986**, *375*, 359–367.

SAMPLE
Matrix: blood
Sample preparation: 0.3-1 mL Plasma + 2 μg phenacetin in MeOH, add to a Sep-Pak C18 SPE cartridge, wash with 7.5 mL water, elute with 5 mL MeOH, elute with 5 mL EtOH, evaporate the eluate, reconstitute with MeOH, inject an aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 Cosmosil-10-phenyl (Nacalai Tesque)
Column: 100 × 4.6 Cosmosil-5-phenyl (Nacalai Tesque)
Mobile phase: MeCN:50 mM pH 5.0 sodium acetate buffer 23:77
Flow rate: 1
Detector: UV 277

CHROMATOGRAM
Retention time: 11.3

Internal standard: phenacetin (8.9)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; plasma; pharmacokinetics; SPE

REFERENCE
Sakamoto,K.; Nakamura,Y. Stereoselective disposition and metabolism of pinacidil in rat, *Xenobiotica*, **1994**, *24*, 329–338.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 3-4 mL Plasma or 2 mL urine to a Sep-Pak C18 SPE cartridge, wash with 7.5 mL water, elute with 5 mL EtOH, inject an aliquot of the plasma extract. Purify the urine extract using a Silicagel 60 F254 (Merck) TLC plate with dichloromethane:EtOH as solvent 5:1.

HPLC VARIABLES
Guard column: 30 × 4.6 Chemcosorb-7-ODS-L (Chemco)
Column: 150 × 4.6 Chemcosorb-7-ODS-L (Chemco)
Mobile phase: Isopropanol:EtOH:50 mM pH 2.5 ammonium perchlorate 3:12:85 containing 3% γ-cyclodextrin and 20 mM sodium sulfate
Flow rate: 1
Detector: UV 277

CHROMATOGRAM
Retention time: 8.3 (-), 9.0 (+)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; plasma; pharmacokinetics; SPE; chiral

REFERENCE
Sakamoto,K.; Nakamura,Y. Stereoselective disposition and metabolism of pinacidil in rat, *Xenobiotica*, **1994**, *24*, 329–338.

SAMPLE
Matrix: microsomal incubations, urine
Sample preparation: Urine. Add 0.1-1 mL urine to a Sep-Pak C18 SPE cartridge, wash with 7.5 mL water, elute with 5 mL MeOH, elute with 5 mL EtOH, evaporate the eluate to dryness under reduced pressure, reconstitute in MeOH, inject an aliquot. Microsomal incubations. 3 mL Microsomal incubation + 2 mL water + 50 μL 200 μg/mL phenacetin in MeOH, homogenize. Remove a 2 mL aliquot and add it to 1 mL 10% perchloric acid, centrifuge at 3000 rpm for 5 min, add the supernatant to a Sep-Pak C18 SPE cartridge, wash with 7.5 mL water, elute with 5 mL MeOH, elute with 5 mL EtOH, evaporate the eluate to dryness under reduced pressure, reconstitute in MeOH, inject an aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 Cosmosil-10-phenyl (Nakarai Tesque)
Column: 150 × 4.6 Chemcosorb-7-ODS-L (Chemco)
Mobile phase: MeCN:MeOH:50 mM pH 5.0 sodium acetate buffer 15:20:65
Flow rate: 1
Detector: UV 277

CHROMATOGRAM
Retention time: 17.6

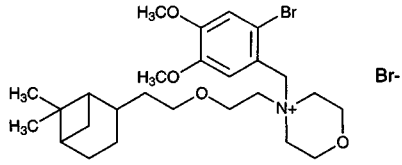
Internal standard: phenacetin (11.32)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; SPE; liver; rabbit; dog; mouse; monkey; human

REFERENCE
Sakamoto,K.; Nakamura,Y. Urinary metabolites of pinacidil: I. Isolation and identification of the metabolites in rat urine, *Xenobiotica*, **1993**, *23*, 391–400.

Pinaverium bromide



Molecular formula: $C_{26}H_{41}Br2NO_4$
Molecular weight: 591.42
CAS Registry No.: 53251-94-8
Merck Index: 7595

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 213.4

CHROMATOGRAM
Retention time: 21.337

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.